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Deciduoma Formation in Pseudopregnant Rats Bearing Pituitary Grafts

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ABSTRACT—Decidual cell reaction was investigated in the uterus of adult rats of the T strain receiving a single pituitary gland each under the renal capsule at 3 months of age. The females with the grafts invariably showed repetitive pseudopregnancies characterized by prolonged, diestrous vaginal smears having copious mucus and activated corpora lutea in the ovaries. The mean length of vaginal cycles was 16.3 and 18.7 days in the pituitary-grafted rats until ovariectomy at ages of 4 and 5 months, respectively. The pituitary grafts equally showed a marked immunohistochemical staining of prolactin in all experimental groups. The decidual response to uterine traumatization was markedly reduced in the pituitary-grafted rats given a 7-day treatment with progesterone alone or in combination with a small amount of estrogen commencing after ovariectomy. By contrast, the control rats bearing isografts of submaxillary glands exhibited 4-day cycles regularly, and always responded positively to the trauma, forming massive deciduomata. These findings indicate that the repetitive pseudopregnacies result in a decline in the uterine reactivity to trauma in the pituitary-grafted rats. Ovariectomy at the time of grafting greatly elevated incidence of deciduomata in the pituitary-grafted rats. The present study suggests that the lowered deciduogenic ability of the pituitary-grafted rats exhibiting the repetitive pseudopregnancies is largely ascribable to the continued exposure of the uterus to ovarian steroids, progestin in particular, rather than to prolactin secreted from the pituitary grafts.

INTRODUCTION

The declined reproductive function in aged female animals is thought to be primarily due to the age-related changes in the central nervous system which controls the gonadal functions in female laboratory rodents [1–8]. However, several authors have suggested that a deficiency in uterine ability to induce decidual cell reaction in aged rodents is, at least in part, responsible for the reproductive malfunction in aging females [9–12]. It has been demonstrated in the middle-aged rats that the age-related decline in decidual cell reaction in response to artificial endometrial stimulation is closely related to the occurrence of persistent vaginal estrus [13]. This is in good agreement with the results in persistent estrous rats produced by neonatal administration of gonadal steroids, suggesting that their uteri are affected by continued exposure to endogenous estrogen as adults [14–16].

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Repetitive pseudopregnancies, characterized by prolonged diestrus and activated corpora lutea in the ovaries, likwise occur spontaneously in rats at middle age [13, 17]. It is known that the aged rats undergoing repetitive pseudopregnancies exhibit a lowered decidual response comparable to the persistent-estrous females [13]. However, the possible effects of repetitive pseudopregnancies on the uterine function have not been duly studied. On the other hand, pseudopregnancy can be induced in rats by transplantation of the anterior pituitary into the subrenal-capsular space [18]. In the present study, the uterine capacity of deciduoma formation was investigated in rats bearing the pituitary grafts in order to determine whether or not repetitive pseudopregnant states interfere with uterine function in adults.

MATERIALS AND METHODS

Virgin female rats of T strain were raised in a temperatureand light-controlled room ($22\pm2^{\circ}C$, lights on from 0500 to 1900 hr). Standard labora-

tory chow (Clea Co., Tokyo) and water were given to the animals *ad libitum*. Daily vaginal smears were taken from at least 1 month before the start of experiment. In the first experiment, 18 rats received a single pituitary gland, obtained from their female litter-mates, each under the left renal capsule on the second day of diestrus after 3 months of age. Nineteen rats likewise receiving isografts of submaxillary glands served as controls. The pituitary-grafted and control rats were assigned to 2 groups, respectively, and ovariectomized on the day of diestrus at ages of 4 months (Group Ia and Group IIa) and 5 months (Group Ib and Group IIb). The rats were then injected s. c. with 3 mg progesterone (P, BDH Labs., Poole, Dorset, U.K.) in 0.1 ml sesame oil for 7 consecutive days commencing on the day after ovariectomy.

In the second experiment, 36 females likewise receiving grafts of pituitary or submaxillary glands at 3 months of age were ovariectomized 2 months after grafting. These pituitary-grafted and control rats were then divided into 2 groups each (Groups Ic and d, and Groups IIc and d, respectively) and given either a single (Groups Ic and IIc) or 7 consecutive injections of 0.1 μg estradiol-17β (E_2, Sigma) in 0.05 ml oil (Groups Id and IId), simultaneously with 7 daily injections of 3 mg P starting on the day following ovariectomy. A single injection of E_2 was performed on the third day of the injection period of P. In the third experiment, 2 groups of 9 rats were ovariectomized at the time of grafting of the pituitary (Group Ie) or submaxillary glands (Group IIe) at 3 months of age. They were given 3 daily injections of 0.1 μg E_2 in 0.05 ml oil prior to the commencement of the P injection at 5 months of age.

On the 4th day of the 7-day injection period of P alone or in combination with E_2, endometrium of the right uterine horn of each rat was scratched along the entire length by a needle with a bent point inserted into the uterine lumen via a small incision made near the cervical end of the horn [19]. On the day following the last injection, the animals were sacrificed. After uteri were checked for gross evidence of deciduomata, the stimulated and intact uterine horns of each animal were weighed separately and fixed in Bouin's solution. The magnitude of decidual cell reaction (DCR index) was estimated by percent increase in weight of the stimulated horn over the intact horn [20].

Ovaries were also weighed and fixed together with pituitary-grafts recovered from each animal. Sections were cut in paraffin at 6 μm and stained with Delafield's hematoxylin and eosin. Some sections of the pituitary graft from each animal were stained by the biotin-avidin immunoperoxidase method [21] for demonstration of prolactin cells. Deparaffinized and rehydrated sections were immersed in 0.3% hydrogen peroxide for 10 min and were pretreated with normal goat serum for 10 min. Then, the sections were sequentially exposed to the following solutions at room temperature: 1:5000 diluted rabbit anti-rat prolactin serum (3 hr), supplied by Institute of Endocrinology, Gunma University, biotinylated goat anti-rabbit IgG (20 min), and avidin-biotinylated horseradish peroxidase complex (20 min) (Imummo-histochemical staining kit, Biomeda Co., CA). After each step, the sections were washed with phosphate buffered saline. The chromogenic reaction was developed by incubating the sections in a solution containing 0.05% 3′,3′-diamino-benzidine 4 HCl and 0.01% hydrogen peroxide in 0.05 M Tris buffer. The data were statistically analyzed by Student's t test and Fisher's exact probability test.

RESULTS

Female rats receiving a single pituitary graft on the second day of diestrus after 3 months of age showed vaginal smears of proestrus on the next day of the grafting and then prolonged diestrous smears accompanying copious mucus. The prolonged diestrus was interrupted by proestrus at which the smears were occasionally mixed with cornified cells. They repeated the prolonged diestrus during a period of 1 or 2 months until ovariectomy. The mean length of vaginal cycles, from the initial to the terminal diestrus, was 16.3 ± 0.8 and 18.7 ± 0.8 days in the pituitary-grafed rats ovariecomized at ages of 4 and 5 months, respectively. The ovaries removed from the pituitary-gratfed rats invariably contained numbers of large corpora lutea (Fig. 1). They were significantly heavier than in the age-matched, regularly cycling controls

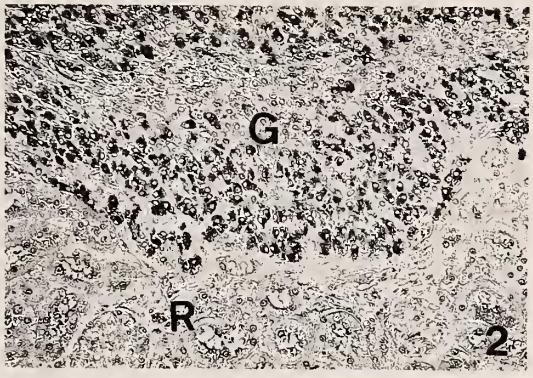

FIG. 1. Ovary from a rat with a pituitary graft under the renal capsule for 2 months after transplantation. Note the presence of a number of large corpora lutea. ×12.

FIG. 2. Pituitary graft recovered 2 months after transplantation. Note marked immunohistochemical staining for prolactin. G: graft, R: renal tissue. ×130.

TABLE 1. Deciduoma formation in the pituitary-grafted and control rats given injectons of progesterone or progesterone plus estrogen

Group	Age at[a] ovariectomy	Positive response	Weight (mg)[b] of induced horns	Weight (mg)[b] of intact horns	DCR index (%)	Weight of ovaries (mg)
Pituitary-grafted						
Ia	4M	0/10**	(121.4± 5.7)	80.1±2.8*	—	83.4±2.6**
Ib	5M	1/9**	173 (118.5± 5.9)	90.6±3.5*	100	83.7±1.2**
Ic	5M	2/9**	306, 527 (121.3± 3.8)	85.0±4.0*	233, 584	83.8±2.1**
Id	5M	0/9**	(162.0± 9.1)	116.3±4.0**	—	83.8±2.3*
Ie	3M	9/9	221.6±38.4	48.4±1.4	381.2±77.8	74.2±2.7
Control						
IIa	4M	9/9	446.0±23.0	89.2±1.7	415.7±31.8	70.2±2.1
IIb	5M	9/9	478.9±50.6	99.1±2.1	392.7±53.6	75.6±2.3
IIc	5M	9/9	665.9±36.6	96.8±2.1	613.9±43.8	75.8±2.5
IId	5M	9/9	944.9±68.6	134.6±3.9	600.0±45.7	76.7±1.8
IIe	3M	9/9	218.1±39.0	46.3±1.4	360.8±73.5	72.9±3.2

All groups of rats received grafts of pituitary or submaxillary glands (control) at 3 months of age. Groups Ia and b, and Groups IIa and b were given 7 daily injections of 3 mg progesterone (P) after ovariectomy. Groups Ic and IIc were given a single injection of 0.1 μg estradiol-17β (E_2) on the 3rd day of the injection period of P. Groups Id and IIa were given 7 daily injections of 3 mg P plus 0.1 g E_2. Groups Ie and IIe were given a 3-day priming with 0.1 μg E_2 prior to the commencement of P injections. The mean weight of stimulated deciduoma-free horns is given in parentheses.

[a] Month.

[b] Mean±Standard error.

* $P<0.05$ and ** $P<0.01$, significance of differences from the corresponding controls.

bearing grafts of submaxillary glands, (Table 1). The pituitary-grafts recovered after approximately 1- or 2-month existence under the renal capsule were always well vascularized with well opened sinusoids. A majority of cells in the grafts showed a marked immunohistochemical staining of prolactin in all experimental groups (Fig. 2).

The control animals invariably formed de-

ciduomata in response to the endometrial traumatization applied at the 4th P injection, when ovariectomized at 4 (Group IIa) or 5 months of age (Group IIb) (Table 1). By contrast, the uterine ability to form deciduomata following similar schedule of treatment was greatly reduced in the pituitary-grafted rats (Groups Ia and Ib) repeating prolonged diestrus regardless of age difference at ovariectomy performed 1 and 2 months after grafting. The intact horns were always smaller in the pituitary-grafted rats than in the controls.

In rats given a single (Group IIc) or 7 daily (Group IId) injections of a small amount of E_2, together with the standard schedule of P treatment for uterine sensitization, the uterine sensitivity to trauma was greatly increased in the control animals ovariectomized at 5 months of age. The magnitude of decidual response as estimated by the DCR index was significantly larger in these 2 groups of control rats given P plus E_2 than in those given P alone (Group IIb) ($P<0.01$). Among the control rats given P plus E_2, repeated injections of E_2 produced larger deciduomata in the stimulated horns, as compared with the single injection ($P<0.01$). However, since the contralateral intact horns were always heavier in the controls given E_2 consecutively than in those given singly ($P<0.01$), the difference in the DCR index was statistically nonsignificant between these 2 groups of control rats ($P>0.8$). In the pituitary graft-bearing rats ovariectomized at 5 months of age, a single or repeated injections of E_2 in combination with the standard schedule of P treatment were incapable of elevating the uterine ability to form deciduomata. The incidence of the response was approximately the same among 3 groups of the pituitary-grafted rats receiving one of 3 kinds of treatments, i.e. 2 different modes of injections of P plus E_2 (Groups Ic and d) and injections of P alone (Group Ib) ($P>0.2$, respectively). In the pituitary-grafted rats, as well as the control animals, the repeated injections of E_2 together with P elicited a significant increase in weight of the intact uterine horn (Group Ib vs Group Id, $P<0.01$), although the horn weight in the pituitary-grafted rats (Group Id) was usually smaller than in the controls given the similar treatment of P plus E_2 (Group IId).

Two groups of rats ovariectomized at the time of grafting of the pituitary (Group Ie) or submaxillary glands (Group IIe) at 3 months of age showed diestrous vaginal smears with many leukocytes until the 3-day E_2 priming prior to the standard schedule of P treatment commencing at 5 months of age. Either the last day of the priming or the next day, vaginal smears typical of proestrus or estrus were observed in both the pituitary-grafted and control rats. During the 2-month postoperative interval, the genital tract underwent progressive atrophy in these two groups of animals. The mean final weights of the intact horns in these groups were significantly smaller as compared with their age-matched groups given similar injections of P from the day after ovariectomy (Groups Ib and IIb) ($P<0.01$, respectively). These pituitary-grafted rats always formed deciduomata in response to trauma, contrasting strongly with those ovareictomized at 5 months of age. The incidence and magnitudes of deciduomata were approximately the same in these two groups of the pituitary-grafted and control rats ovariectomized simultaneously with the grafting.

DISCUSSION

In female laboratory rodents, anovulatory sterility exhibiting persistent vaginal estrus and repetitive pseudopregnancies characterized by prolonged diestrus resulting from activation of corpora lutea in the ovaries is an aging phenomenon of the reproductive system [13, 17]. In younger animals, however, pseudopregnancy can be induced by such procedures as a single injection of reserpine on the first day of diestrus [22], sterile copulation [23], mechanical stimulation of the uterine cervix during estrus [24] and grafting of the anterior pituitary gland [18]. Everett [25] has demonstrated that the luteal function continues for as long as 3 months in rats receiving autografts of the anterior pituitary gland. In the present study, female rats bearing pituitary grafts under the renal capsule repeated prolonged diestrus accompanying copious mucus and activated corpora lutea in their ovaries 1 or 2 months after the grafting. The difference in luteal activity and the resultant diestrus between the previous and present studies may

be due to difference either in auto- and isografts or in criteria of smear observations. Histological and immunohistochemical studies suggested prolactin secretion from the pituitary grafts regardless of the duration of subcapsular pituitary existence in the kidney.

The decidual cell reaction in response to uterine traumatization applied during the 7-day injections of P was definitely reduced in two groups of the pituitary-grafted rats examined 1 and 2 months after grafting as compared with that in the age-matched controls grafted with submaxillary glands. A single injection of E_2 mimicking a "nidatory surge of estrogen" or repeated injections of E_2, together with P injections, failed to increase uterine sensitivity to trauma in the pituitary-grafted rats, while in the control rats, the treatment of E_2 in combination with P always produced greater decidual response, as compared to the treatment with P alone. Changes in the uterine response to trauma in the ovariectomized rats bearing pituitary isografts are similar to those in the hypophysial-autotransplanted rats with their ovary intact. In these animals, the uterine response began to lower approximately 1 month after the grafting [26]. These findings indicate that the repetitive pseudopregnancies result in a low uterine reactivity to the deciduogenic stimulus in the pituitary-grafted rats as well as in aged rats [13].

It is known that the continued exposure of uterus to endogenous estrogen is responsible for the reduction of deciduogenic ability in aged and androgen-sterilized rats exibiting persistent estrus [13, 27, 28]. The present study revealed that the uteri of the pituitary-grafted rats were sensitive to trauma if the repetitive pseudopregnancies were inhibited by ovariectomy at the time of the grafting. The uterine response was approximately the same in both incidence and magnitude in the pituitary-grafted and control rats whose ovaries were removed at the grafting. Accordingly, the lowered uterine sensitivity to trauma in the pituitary-grafted rats undergoing the repetitive pseudopregnancies may be largely ascribable to the effect of the continued exposure of the uterus to ovarian steroids, progestin in particular, rather than to prolactin secreted from the pituitary grafts. From these findings, it seems unlikely that the increase in circulating prolactin levels with age [29] is responsible for the age-related decline in decidual cell reaction.

Decidual reaction has been used as the test for luteal function in females receiving various procedures which are effective in eliciting pseudopregnancy [18]. The present study, however, represents that deciduoma formation is not always reliable, at least in a long-term experiment, as a criterion for the luteal function in the pituitary-grafted rats.

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